AN IMPRESSION METHOD FOR PREPARING FISH SCALES FOR AGE AND GROWTH ANALYSIS

Edgar L. Arnold, Jr.
U. S. Fish and Wildlife Service
Woods Hole, Massachusetts

SINCE CONCENTRIC rings on fish scales were first recognized as being indicative of growth, scale study has become an increasingly important part of fishery investigation. Before scales from most species of fish are studied, they are mounted for observation under a microscope or some other magnifying apparatus.

This paper deals with procedures in preparing fish scales for analysis and describes in detail the method used for a number of years by the North Atlantic Fishery Investigations of the Fish and Wildlife Service. The incentive to write the paper was provided by numerous inquiries received through the mail and by the interest evinced by many fishery biologists visiting the headquarters at Woods Hole, Massachusetts.

REVIEW OF REPRESENTATIVE METHODS

Mounting without Medium

Temporary Mounts

Scales are arranged on a transparent slide; where illumination involves heat, often a cover slip is added to prevent curling of the scales. This is the simplest process of preparing fish scales for examination. This method has been adequate for studying scales of haddock up to 3 or 4 years of age and has been satisfactory for scale studies of many other species.

Mounting with Medium

Temporary Mounts

Scales may be immersed in a clear liquid medium (water, glycerin, etc.) before examination. The liquid aids in keeping scales flat and permits them to be moved around deftly with a dissecting needle--without "snapping" out of the field, as frequently occurs in dry mounts.

Permanent Mounts

Scales may be placed within a transparent liquid matrix (balsam, Karo, waterglass and glycerin, or a plastic such as Clarite) that subsequently hardens into a permanent mount. Before immersion, selected scales (those having no regenerated centers) should be carefully cleaned and sometimes stained to bring out certain structural elements. Clarite, a hydrocarbon resin, in a mixture of 60 percent Clarite to 40 percent toluene (by weight) remains clear indefinitely, unlike the other media that tend to whiten with age and thus impair legibility. This is a comparatively slow and tedious process that is still widely used whenever it is

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This material appeared in Physis; Revista de la Asociacion Argentina de Ciencias Naturales, in 1950.

See also:

Lewis, W. M., and Carlander, K. D. 1949. A simple method of mounting scales. Prog. Fish-Cult. 11 (4): 263.

desirable to have the whole scale available for study of internal structures, or where scales are too large or soft to give good results by impression.

IMPRESSION METHOD

In the foregoing methods, mounting consisted of preparing the actual scale for analysis. It has been found that scales from older fish of many species are extremely difficult, and in some cases impossible, to read when mounted by these techniques. This is due primarily to the thickening of the scale with age and a corresponding increase in light refraction so that growth of early years often is entirely obscured. This problem was overcome to a large extent when Lea (Lea and Went 1936) in 1918 proved in regard to herring scales that:

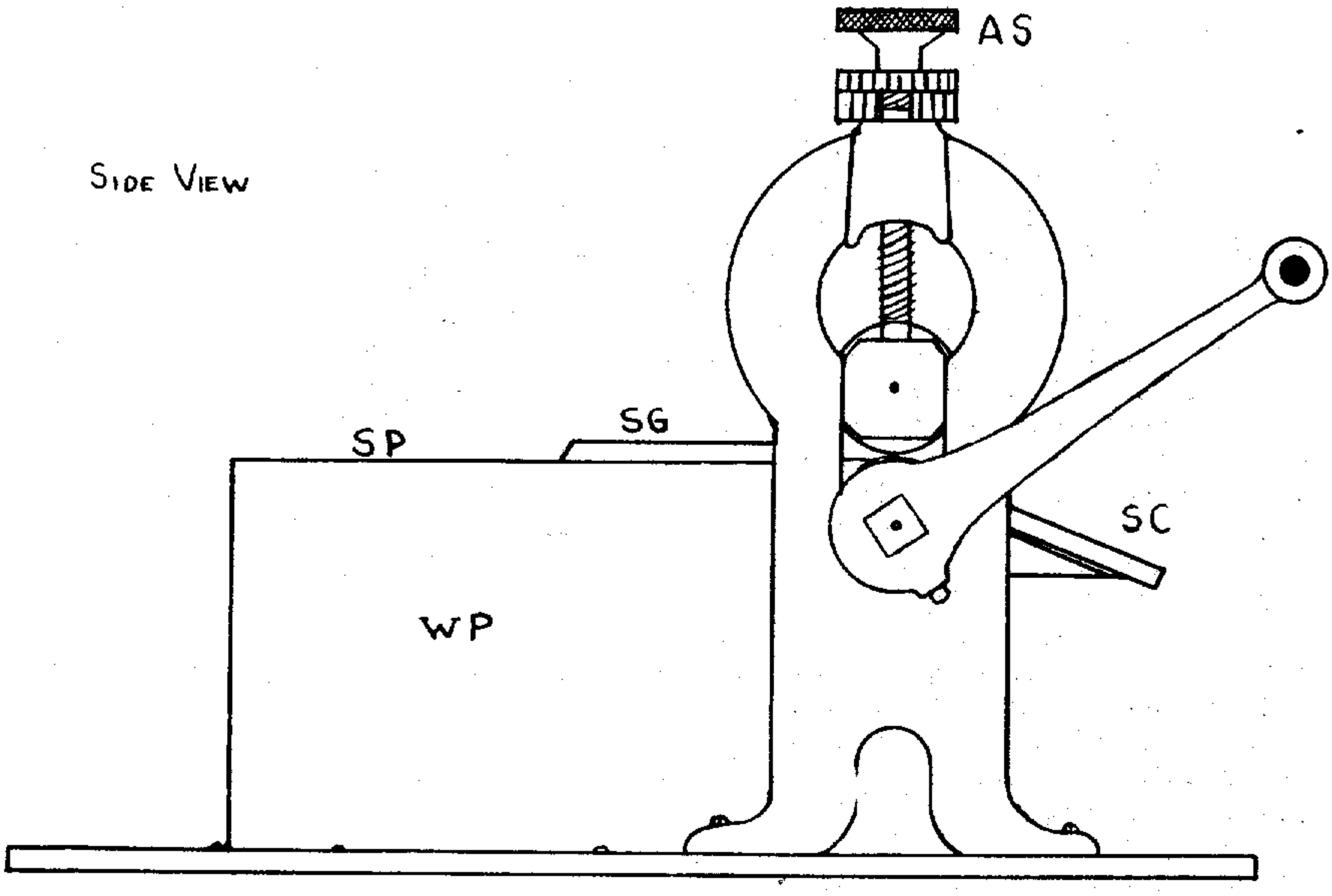
> "... all details which are subjected to observation when the scales are used for the purpose of age determination and growth calculations, arise from the play of light on the delicately moulded relief forming the outer surface of the scales. The structural elements below the surface and on the inner surface of the scales are of no direct interest in the practice of scale analysis."

Lea determined this by making casts of the sculptured surface in thin celloidin films. His comparison of these casts with the original scale revealed that the imprints were as good as the originals in analysis for age and growth--and sometimes better. The celloidin films were delicate, however, and necessitated immediate examination or copying by photography. Recognizing the need for permanency and the value of making copies of scale details for other interested investigators, Lea and Went developed a method that entailed pressing the sculptured surface of the scale against the softened surface of a celluloid plate (solvent consisted of 3 parts of pure redistilled acetone, 1 part of amyl acetate), which gave a negative relief of the scale surface, as in the celloidin film. The celluloid impressions were then filled with a gelatine sol (880 cubic centimeters of water, 120 grams of pulverized gelatine, 20 grams of glycerin warmed gently to about 50° C.), which upon hardening resulted in a fairly durable film bearing positive copies. It was possible to make as many of these "positives" as were desired. Lea and Went further determined that the technique was satisfactory not only for herring but also for many other species, including the Gadidae group, mackerel, salmon, and rosefish. Impressions were made either by means of a 20-minute process in a hydraulic press, or by use of carpenter's clamps, requiring approximately 2½ hours. A number of copies could, of course, be made in one operation.

Nesbit (1934) used a similar procedure to study scales of the squeteague (Cynoscion regalis). Nesbit softened his celluloid plates either by moistening them slightly with alcohol or by exposing them to acetone vapors for several minutes. His impressions were made with a seal press of the type used by notaries, the dies being smooth and highly polished. Best results were obtained with clean, dry scales.

Though Nesbit could readily obtain positive replicas "by pressing the dry scales into heavy lead foil and allowing a few drops of a viscous solution of celluloid in acetone to dry in the depression," he concluded that they were no more desirable for ordinary purposes than mirror-image impressions and were less convenient to store and examine.

After considering and trying existing methods, and finding all of them inadequate in some aspects, the Fish and Wildlife Service's North Atlantic Fishery Investigations developed a modified impression procedure for the preparation of the extensive collection of haddock (Melanogrammus aeglefinus) scales for examination. In this procedure, cellulose nitrate was first used as the impression medium. Two innovations improved the original process: use of a jeweler's type of roll press (figure 1), and softening of the plastic slide by heating on a warming platform connected directly to the press. The platform is made of



AS - Adjusting screws
WP - Warming platform
SP - Surface of WP
SC - Slide catching tray
SG - Slide guide
G - Roller gears
RS - Roller surface (upper)
LS - Light switch

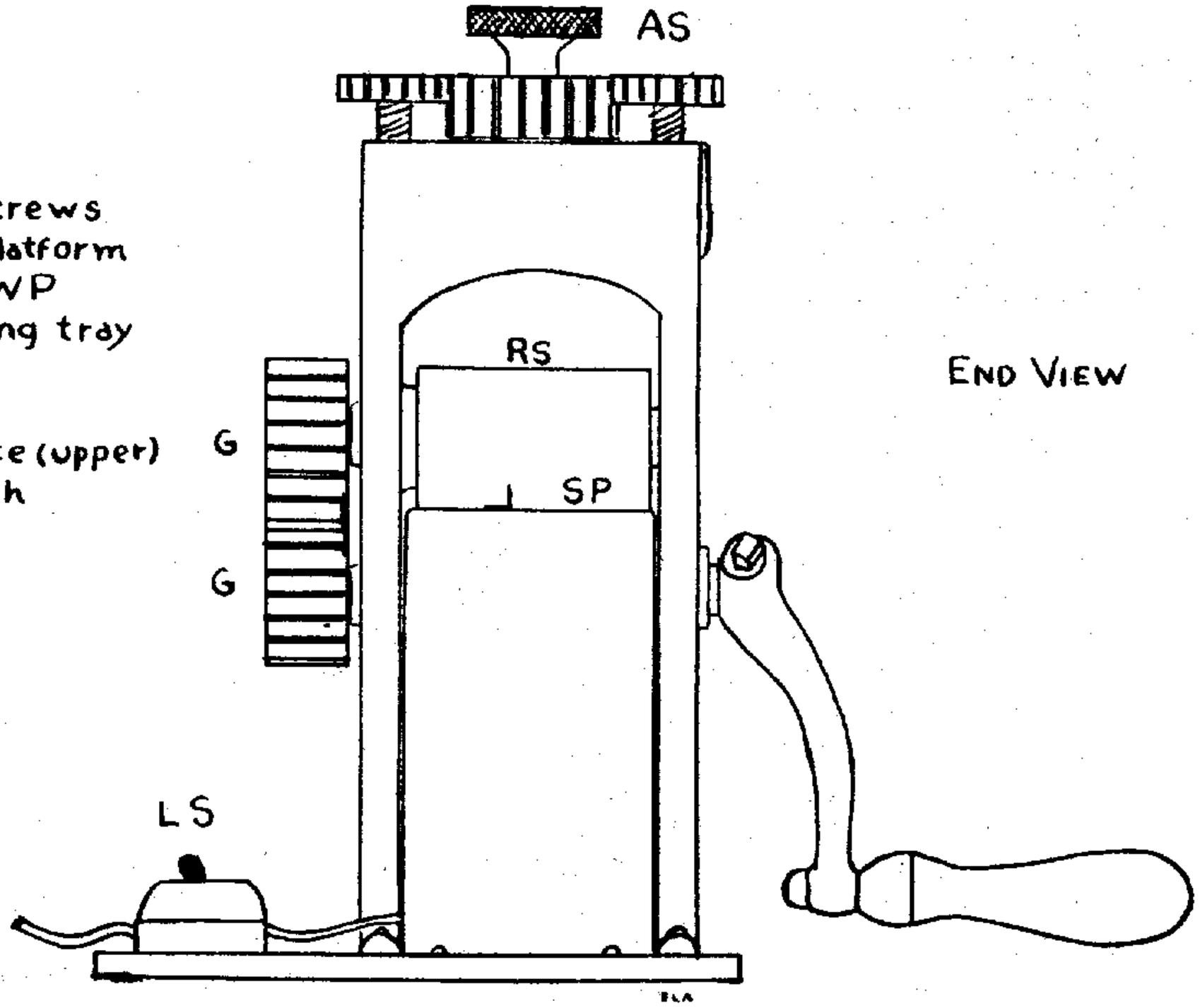


FIGURE 1.--The roll press.

sheet copper to house the heat source, a common 20-watt light bulb, and is attached to the baseboard of the press, the end of the warming surface extending nearly to the rollers. Slides may be moved along this surface and into the rollers without disturbing the alignment of the scales. Softening of the cellulose nitrate by warming eliminates treatment by volatile and highly inflammable chemicals--a decided advantage. The rollers of the press are readily adjustable for different thicknesses of slides, so that it is possible to make impressions of the scales of various species.

When the cellulose-nitrate impressions were stored for an appreciable period, difficulties were experienced. Chemical reactions, for instance, caused bad discoloration and cracking. Handling of such slides often resulted in their disintegration. Furthermore, because the plastic was highly combustible, it was necessary to store the slides in a basic aqueous solution. These difficulties were overcome by the adoption of another plastic material, cellulose acetate, as an impression medium. Relatively fireproof, unbreakable, and very durable, this material has proved most satisfactory (figure 2).

Sheets of the plastic, 0.02 inch in thickness, are cut into slides with a photographic print cutter. Dimensions of slides depend on the size of the scale and the method of storage. For haddock scales, $2\frac{1}{2}$ by $\frac{1}{2}$ inch was chosen as the most suitable size. Each slide is prepared for mounting by inscribing abbreviated pertinent data, i.e., the serial number, area where fish was caught, and length of fish, on the right-hand end. Labeling is done with a special black opaque ink that is indelible, waterproof, and has the property of etching the plastic surface--because of its acetone content--and thus cannot be rubbed off. The ink requires several hours to dry.

Clean scales are a prerequisite for clear, legible impressions. In haddock work, this condition is very easily met by scraping body slime and foreign matter from the surface of the fish before taking scales. The scales (15 to 30) are then placed between sheets of thin blotting paper in a small coin envelope.

For the actual mounting or impressing routine, six or seven scales which do not have regenerated centers are selected from each scale envelope. These scales are placed, sculptured surface downward, on the slide bearing the same serial number. The edges of most scales tend to curl in the direction opposite the sculptured surface when correctly placed; in others, the sculptured surface can be determined by gentle stroking with a dull needle. A microscope or dissecting-scope is unnecessary for these operations, although a hand lens may be useful for examining very small scales. For uniformity and easier reading, the anterior radii of the scales, usually the broader ends, are pointed toward the top of the slides. The slide is then placed on the warming platform, labeled end toward the rollers, for approximately 10 seconds. The now slightly softened slide is run through the rollers of the press. The scales usually drop off in the process, but some adhere to the rollers. Constant care should be exercised, therefore, to keep rollers free of scales so that contamination of succeeding mounts will not occur. Impressions are frequently checked for legibility and proper roller adjustment. Although most of our work has been done with haddock scales, we have found it possible to produce satisfactory impressions from the scales of almost any other species of fish. The proper combination of heat, pressure, and thickness of celuloid (cellulose acetate) is generally easy to determine. Sometimes, as with scales which are thick in the center, an extra celluloid strip on top of the scales results in a better impression. Passage of the slides through the rollers often causes them to curve upwards at the ends. This undesirable effect is corrected by immediately placing each newly made impression, curved surface downward, in a receiving niche cut into a small block of wood and putting a lead weight on top of the mounts.

When a group of 20 slides has been completed, the slides are bundled together and passed on to the analyst. The envelope with remaining scales is stored as insurance against possible loss of impressions.

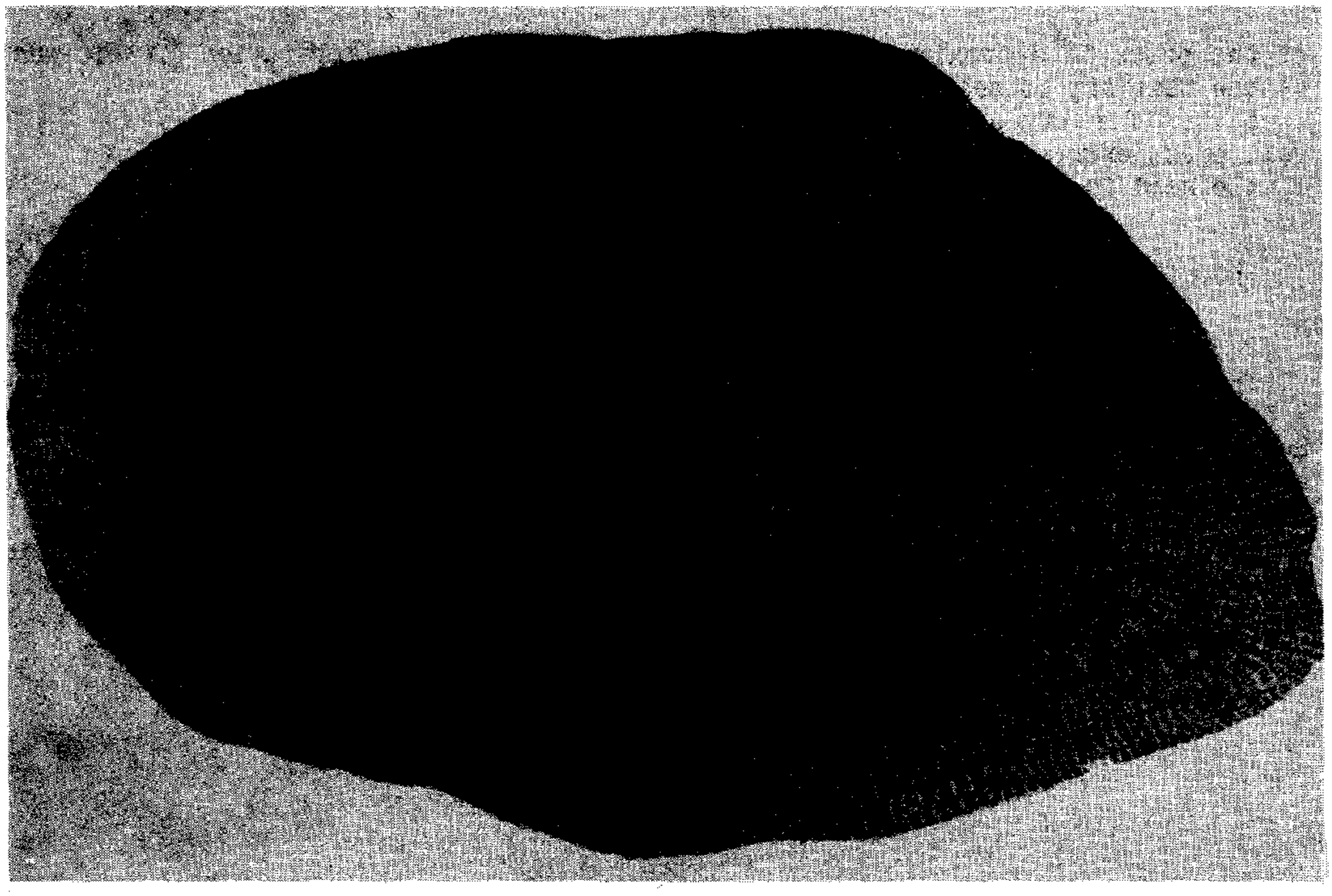


FIGURE 2.--Photograph of an impression made from the scale of a 6-year-old haddock.

To facilitate examination of the impression mounts under a microscope, a holder should be constructed as shown in figure 3. The size of holder would, of course, depend on the size of slide used. The holder should be made of a heavy metal substance, such as brass, with hold-down clamps of any springy metal. If a mechanical stage is available, the plastic mounts can be placed over a glass slide and held down by similar clamps attached to the stage.

Advantages of Impression Method

The advantages of the method for impressing haddock scales, as described, may be summarized as follows:

1. Increased legibility. This is especially true for reading scales of older fish.

2. Less storage space required. Four hundred slides can be stored in the common slide box that holds only 25 glass slides. In our situation, with a collection of some 80,000 haddock scale slides, storage of the glass type would present difficulties.

3. Relative speed of the process. An average of 200 mounts a day can be turned out by a competent technician. This does not include cutting and labeling of the slides.

4. Durability and moderate cost of mounting medium and apparatus. The plastic is practically unbreakable, retains legible impressions indefinitely, and is substantially cheaper than glass--800 2½- by ⅛-inch slides can be cut from a 20- by 50-inch sheet costing about $2.15. The roll press and platform are also durable and relatively inexpensive--the cost

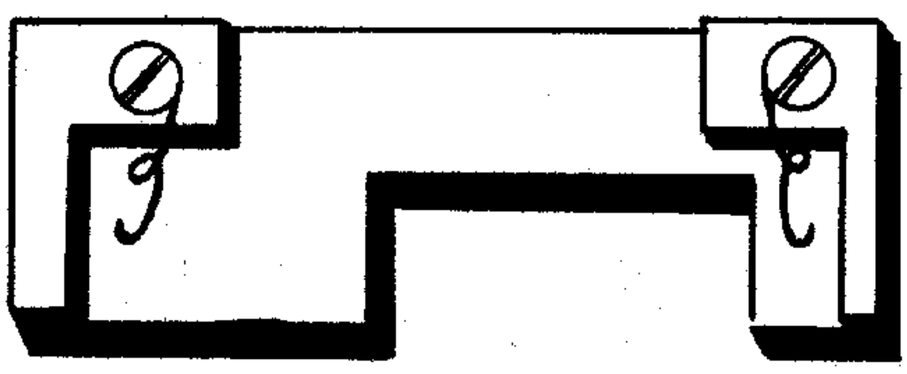


FIGURE 3.--Holder for plastic slides.

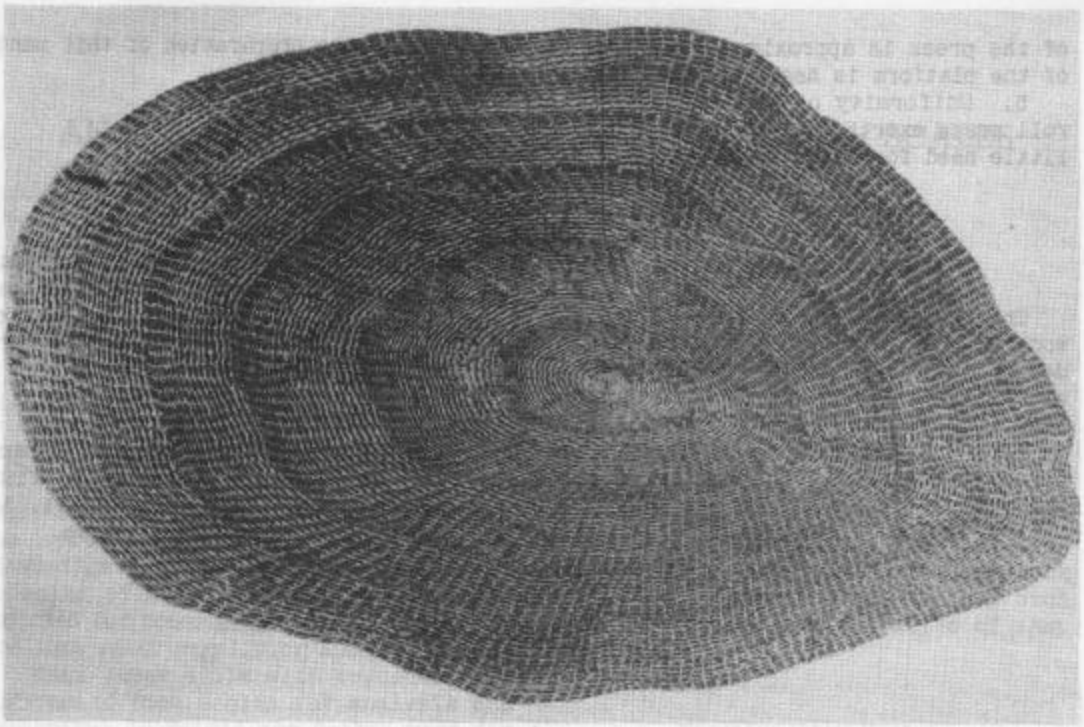


FIGURE 2.--Photograph of an impression made from the scale of a 6-year-old haddock.

of the press is approximately $30; that of the platform is negligible.

5. Uniformity of impressions. The roll press exerts constant pressure with little need for adjustment.

ACKNOWLEDGMENTS

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